ACUTE EFFECTS OF GAMMA RADIATION IN PRIMATES

SCHOOL OF AVIATION MEDICINE
RANDOLPH AIR FORCE BASE, TEXAS

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In an excellent paper on the acute radiation syndrome in man, Gerstner (1) described a dose – survival time curve containing three "plateaus" or dose-independent regions. This curve, reproduced in figure 1, has been estimated for man from clinical data and animal experiments (2-5). These "plateau regions" are ascribed to three different pathogenic mechanisms — death being caused in the low-dose region by hematopoietic depression, in the mid-dose region by gastrointestinal denudation, and in the high-dose region by a failure of the central nervous system.

According to Storer and coworkers (6), in the mouse the median survival time is observed to decrease exponentially with increasing dose starting at about 500 rads. A "plateau" in the median survival time exists from about 1,200 to 10,000 rads, and for doses from about 10,000 to 150,000 rads an exponential decrease is again observed with a possible "plateau" in the range from 80,000 to 120,000 rads.

The Department of Radiobiology, School of Aviation Medicine, USAF, undertook a study of the survival of *Macaca mulatta* monkeys following whole-body exposure to a source of pure gamma radiation to examine the extent and location of dose-independent regions, to observe carefully the clinical and pathologic syndrome produced by radiation, particularly in the less well understood mid- and high-dose regions, and to determine the $LD_{50/30}$ dose.

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This work was accomplished at the School of Aviation Medicine, USAF, Randolph AFB, Tex., at the Radiobiological Laboratory of the University of Texas and the United States Air Force, Austin, Tex., and at the Southwest Research Institute, San Antonio, Tex.

EXPERIMENTAL METHODS

Whole-body doses of Co^{60} gamma radiation were administered using the Co^{60} facility of the Southwest Research Institute (SWRI), San Antonio, Tex. An essentially homogeneous radiation field was achieved by arranging 32 Co^{60} "tapes" in four circles of 8 tapes each on a rigid frame fashioned from small steel rods which placed the tapes on the surface of a sphere (diameter, 36 in.), enclosing the animal to be irradiated. Each tape contained approximately 237 curies of high specific activity Co^{60} in the form of three cobalt discs, 1 cm. in diameter and 1 mm. thick. The source tapes and their

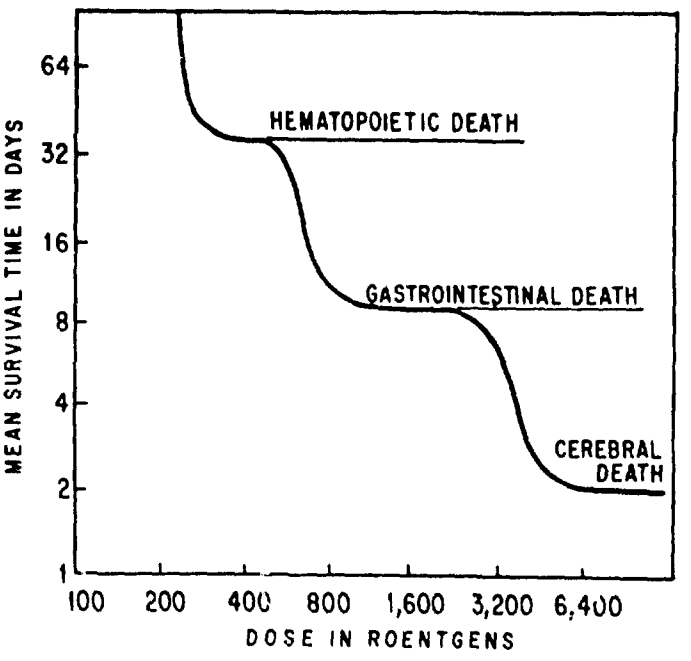

FIGURE 1

Dose – survival time relationship predicted for man.

arrangement are shown schematically in figures 2 and 3. Measurements at the SWRI indicated that there was less than a 10 percent variation in the curie strength for all tapes.

The dose delivered to each animal was governed by the exposure time in the radiation field. Exposures were made by placing the animal in a plastic exposure cage (fig. 4) on a pneumatic lift. The cage and animal were then retracted into an iron- and concrete-shielded well and the source moved from its shielded cell into position beneath the well. The animal

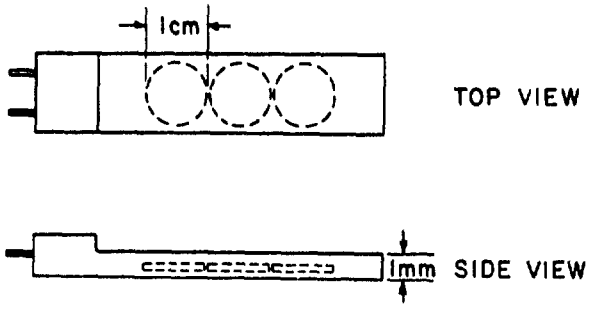

FIGURE 2

Detail of the individual Co" source tapes.

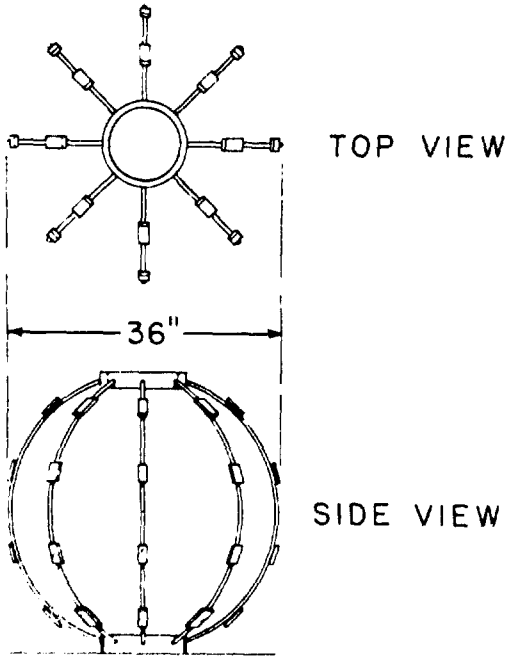

FIGURE 3

Array of 32 sources used to obtain a homogeneous field of high-intensity gamma radiation.

was then rapidly lowered into a predetermined position at the center of the source array (fig. 5). A reverse sequence of events was used to terminate the exposure. The entire sequence of events from the time the source arrived in position underneath the shielded well until the source moved into its shielded cell was automatic with a variable preset timer used to select the exact length of time the animal was positioned at the center of the source array. The "entrance" and "exit" doses (defined, respectively, as the dose received by the animal during positioning of the source and animal, and that received during removal of animal and source) were measured and the reported doses include these dose contributions. The "entrance" and "exit" dose rates were less than the dose rate in the exposure cage during the major portion of the exposure period. This was not considered significant, however, since the sum of the "entrance" and "exit" doses was only 54 r delivered over approximately 10 to 15 seconds, while the main portion of the dose was delivered at a rate of 800 r/minute over periods ranging from about 30 seconds to 50 minutes. The dose range investigated extended from 400 to 40,000 r. Dosimetry, using single-phase trichloroethylene chemical dosimeters, was performed to establish the dose rate prior to exposing animals. In addition, chemical dosimeters were affixed to each animal during exposure, these dosimeters being placed around the waist of the animal—back and front. Depth dose measurements were also made by placing chemical dosimeters at various levels and depths in an animal sacrificed for this purpose and also in a tissue-equivalent phantom.

Continuous clinical observations were made during irradiation and at frequent intervals for a 30-day period following exposure. The prominent clinical signs included hyperirritability, convulsions, ataxia, debility, vomiting, diarrhea, weight loss, erythema, purpura, epilation, and ulcerations.

The error in survival time measurement was of the order of 1 percent or less, autopsies being performed as soon as possible and always within 30 minutes. In addition to the autopsies on these animals progressive pathology was observed on several animals exposed at the

500 and 725 r levels. These animals were serially sacrificed at approximately 2-day intervals.

Postmortem examinations included organ weights, photographs of specimens and microscopic examinations, as well as general observations concerning gross changes apparent at postmortem. Long-term follow-up of survivors is being carried out. The experimental design is given in table I.

The homogeneous group of adolescent animals (age 5 years) used in this experiment was received in a single shipment from overseas. They had previously been held for an observation period in which freedom from disease, including tuberculosis, had been established. The animals ranged in weight from a minimum of 5 pounds to a maximum of 10.25 pounds with an average weight for the group of 7 pounds. The grouping of animals in dose levels and sex was carried out by a system of random selection.

DOSIMETRY

Radiation measurements were made primarily with the Air Force one-phase chemical dosimeter (7). Dosimeters of several ranges were made for this study – that is, 150 to 1,000 r; 400 to 5,000 r; 1,000 to 10,000 r; 10,000 to 60,000 r. All dosimeters were evaluated with a modified Beckman DK-2 spectrophotometer. This evaluation technic is reported in ITR-1500 (Operation Plumbbob).

Four radiation measurements were involved in this study: (1) measurement of the "entrance" and "exit" doses, (2) dose rate measurements, (3) measurement of the dose distribution in the exposure cage, and (4) depth dose measurements.

Two methods were used in measuring the "entrance" and "exit" doses. A direct measurement of the combined "entrance" and "exit" dose was made using three Bendix 100 r total dose ionization chambers. This was accomplished by moving the source into position and inserting and removing the exposure cage, containing the ionization chambers, allowing essentially zero time at the exposure position.

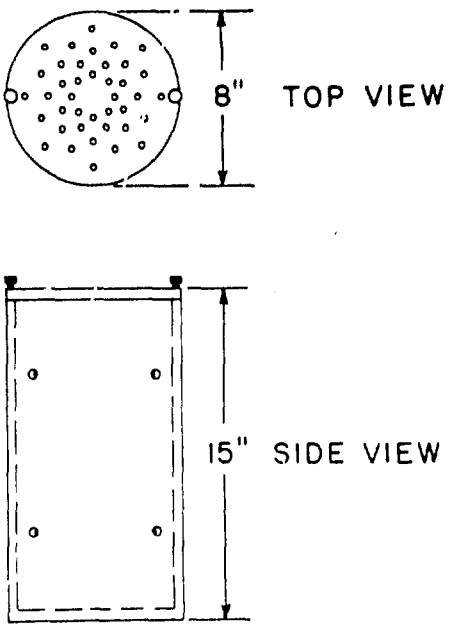

FIGURE 4

Lucite exposure cage.

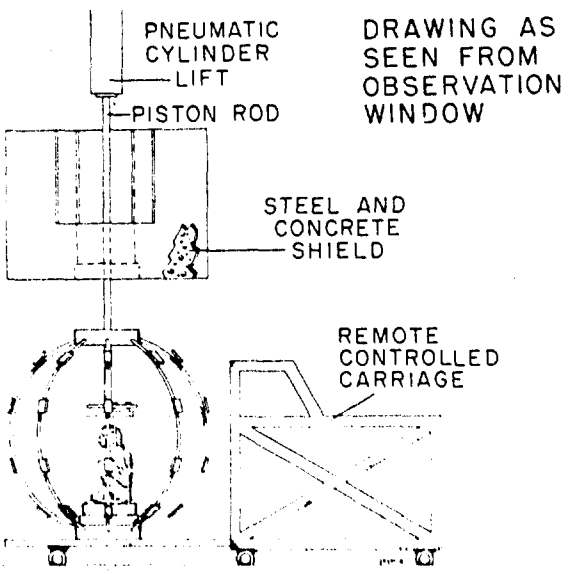

FIGURE 5

Animal in exposure cage within radiation field.

Similarly, a direct measurement of this dose was made using low-range chemical dosimeters by repeating the insertion and withdrawal procedures until the accumulated dose was within the range of the dosimeters utilized. The average dose was then obtained by dividing the total measured dose by the number of insertions. An indirect measure of this combined dose was obtained by measuring the total dose with chemical dosimeters for different times of irradiation and obtaining the zero time dose intercept by least squares fit to the measured data. The results of these measurements are given in table II.

An average dose rate of 800 r/minute was measured in the center of the irradiation cage using several types of dosimeters with varying ranges and used over long exposure periods.

In addition to the dose rate measurements made with the Air Force chemicals, an attempt was made to measure the dose rate with a Brookhaven ionization chamber and electrometer. This system was calibrated previously against the 1,260 curie Co^{60} source at the Radiobiological Laboratory, Austin, Tex. The average dose rate measured in this manner was 920 r/minute. Since the leakage current was not measured, the dose rate of 920 r/minute was only an upper limit and was not considered as representing the actual dose rate.

The accumulated data indicate that the monkeys were irradiated at a dose of very nearly 800 r/minute. Two tests were made to check the symmetry of the dose distribution in air in the exposure cage. Within the accuracy of the dosimeters the data indicated an essen-

TABLE I

Group	Dose (r)	Exposure time (min.: sec.)	Number of animals		
			Male	Female	Total
A	400	00:29	2	3	5
B	500	00.37	4	4	8
C	575	00:42	3	3	6
D	650	00:48	2	4	6
E	725	00:54	5	2	7
F	850	1:03	0	5	5
G	1,500	1:52	2	6	8
H	2,000	2:29	5	3	8
I	2,500	3:07	4	4	8
J	3,500	4:22	5	3	8
K	5,000	6:15	5	3	8
L	7,500	9:23	3	2	5
M	10,000	12:31	1	4	5
N	15,000	18:47	3	2	5
O	25,000	31:19	3	2	5
P	40,000	50:07	1	4	5
*S	500	00:37	0	1	1
*W	725	00:54	0	4	4

*Sacrificed serially.

tially homogeneous radiation field. Figure 6 shows calculated isodose levels throughout the cage volume based on a strength of 237 curies per source tape.

The last measurement was a measurement of the depth dose pattern in a monkey cadaver and in a 6-inch diameter sugar-water-urea phantom. The sugar-water-urea solution consisted of the following substances: 56.9 percent water, 28.4 percent glycerol; 7.6 percent urea; 7.1 percent sucrose. All percentages were measured by weight.

The average dose measured in air throughout the plastic container phantom was 6,150 r for an 8-minute exposure. The doses measured at 1 in., 2 in., 3 in., 2 in., and 1 in., from the outside of the cylindric phantom through the sugar-water-urea solution were 5,510 r, 5,425 r, 5,375 r, 5,250 r, and 5,275 r, respectively. Thus, the depth-dose pattern in the solution was nearly isotropic with a depression in the dose rate equal to approximately 13 percent.

In addition to the dose measurements in the sugar-water-urea solution, radiation measurements were made in a monkey cadaver. The dosimeters were placed in the mouth, the thoracic cavity, posterior to the liver, along the transverse colon, and at the base of the pelvic cavity. The total dose measurements were 6,300 r, 5,250 r, 5,700 r, and 6,300 r, respectively. The cadaver was a male monkey weighing 5¾ pounds. Dosimeters were placed in a belt around the sugar-water-urea phantom and the monkey cadaver as a means of correlating monitored doses for the irradiated monkeys. A depression in this dose, as compared with the air dose, was found, which confirmed our findings in later monitor doses.

With the figure of 800 r/minute for the dose rate and 54 r for the in-out dose, approximate exposure times were calculated for the 107 monkeys exposed. Belts referred to in the preceding paragraph were placed around each animal to monitor each exposure. The same depression in the monitored doses was observed as was found with belts of dosimeters surrounding the phantom, and the monkey cadaver for the higher dose range (i.e., 1,000 to 40,000 r). The monitored doses for the lower ranges of exposures (400 to 850 r) were consistently higher than the predicted doses. It is felt that these higher monitored doses in the lower ranges were caused by a basic calibration error in this particular dosimeter. Unfortunately, it is virtually impossible to check this conclusion since slight changes in the content of a dosimeter solution and its starting pH markedly effect the response of the solution and all of this dosimetric solution was used during this experiment.

TABLE II

Method	Average "entrance"-"exit" dose (r)
Bendix ionization chamber	53
Chemical dosimeters (multiple exposure)	52
Dose intercept	58
Average	54

STATISTICAL ANALYSIS OF DOSE – SURVIVAL TIME RELATIONSHIP

Of 100 animals used in the statistical analysis, 89 died within 30 days after exposure (table III). The times of death could be classified as early, intermediate, and late, thereby dividing the dose-survival curve into three distinct parts. These divisions corresponded closely to the type of death as established by pathologic study and clinical symptomatology. The type of death for animals exposed to 400-1,000 r was classified as hematopoietic; for 1,000 r to 9,000 r as gastrointestinal; and, for 9,000 r to 40,000 r as central nervous system, in accordance with general practice.

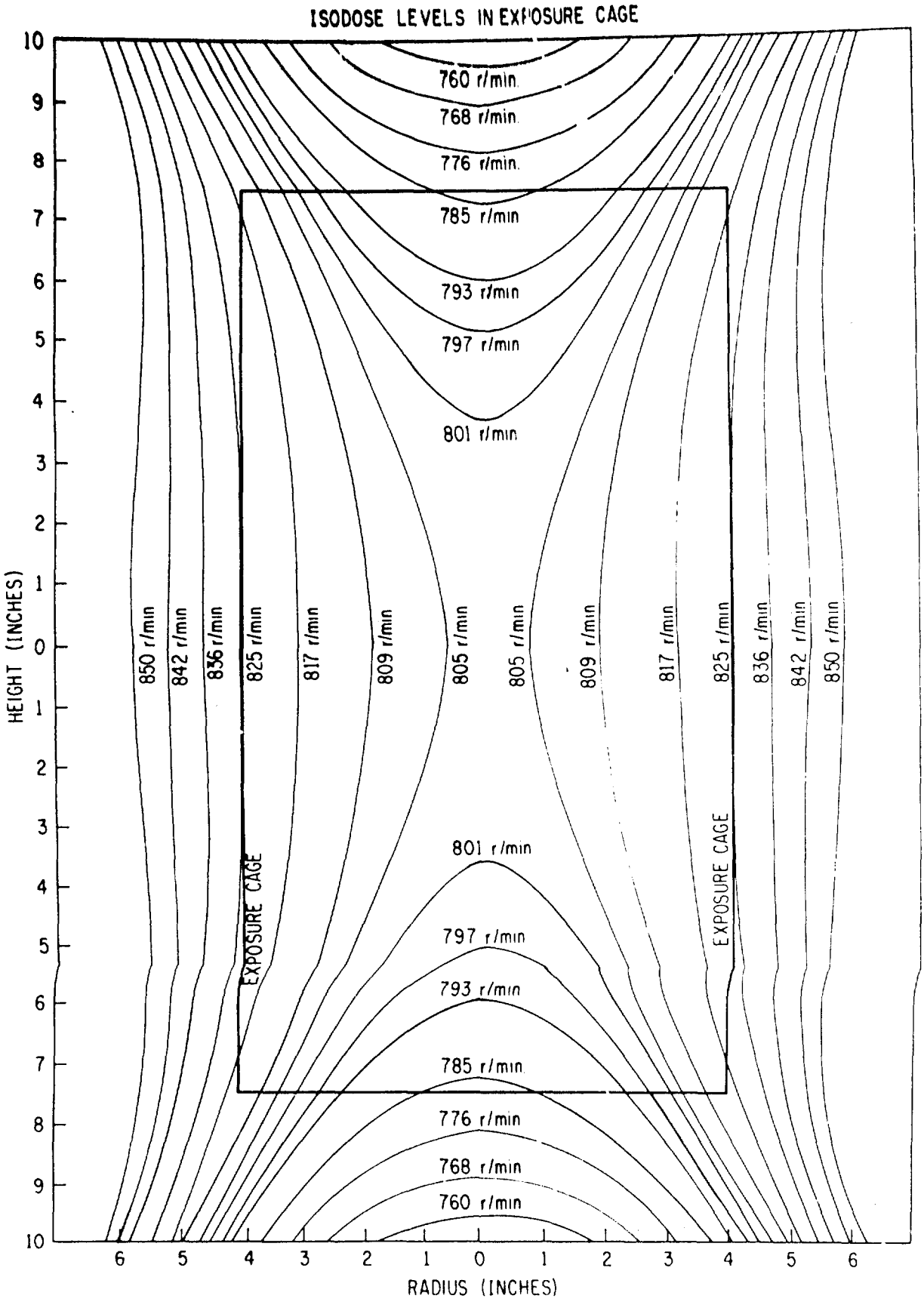

FIGURE 6

Calculated isodose levels in exposure cage neglecting attenuation by cage material.

Because of the distinctive shape of the survival curve, best-fit curves were determined for each individual portion of the curve. Log survival time (ST) in hours, and log dose (D) in roentgens were the units used. Three animals were not included in the analysis; death in two cases was classified as atypical, and the third was omitted in the statistical treatment since the time of death fell outside statistics for that particular dose group. Figures 7, 8, and 9 show the range of times for the individual deaths in each group. The means of each group are given, with the least squares fit to these means drawn in.

The combined log-log plot of survival time versus dose is shown in figure 10. The upper, left-hand graph represents the hematopoietic type of death. Three different curves were fitted to these data. The linear curve

$$\log ST = 3.386 - .3107 \log D$$

is shown as an unbroken line. The exponential curve

$$\log ST = \exp (1.263 - .1218 \log D)$$

is indistinguishable on this plot from the linear curve, in the range from 400 to 1,000 r and was not included. Both of these curves were adequate fits; that is, each curve fitted the data within sampling variability. The mean line (dashed)

$$\text{mean log } ST = 2.520$$

TABLE III

Statistical parameters

Group	Number in group	Number dead in 30 days	Mean survival time (hr.)	Dose (r)	Mean log survival time	Log dose
A	5	1	538	400	2.7312	2.6021
B	8	7	359	500	2.5546	2.6990
C	6	4	287	575	2.4578	2.7597
D	6	2	355	650	2.5499	2.8129
E	6	6	302	725	2.4806	2.8603
F	4	4	331	850	2.5195	2.9294
G	8	8	190	1,500	2.2782	3.1761
H	8	8	150	2,000	2.1758	3.3010
I	8	8	156	2,500	2.1931	3.3979
J	8	8	143	3,500	2.1540	3.5441
K	8	8	142	5,000	2.1524	3.6990
L	5	5	136	7,500	2.1340	3.8751
M	5	5	48	10,000	1.6821	4.0000
N	5	5	13.6	15,000	1.1341	4.1761
O	5	5	6.7	25,000	.8275	4.3979
P	5	5	3.9	40,000	.5951	4.6021

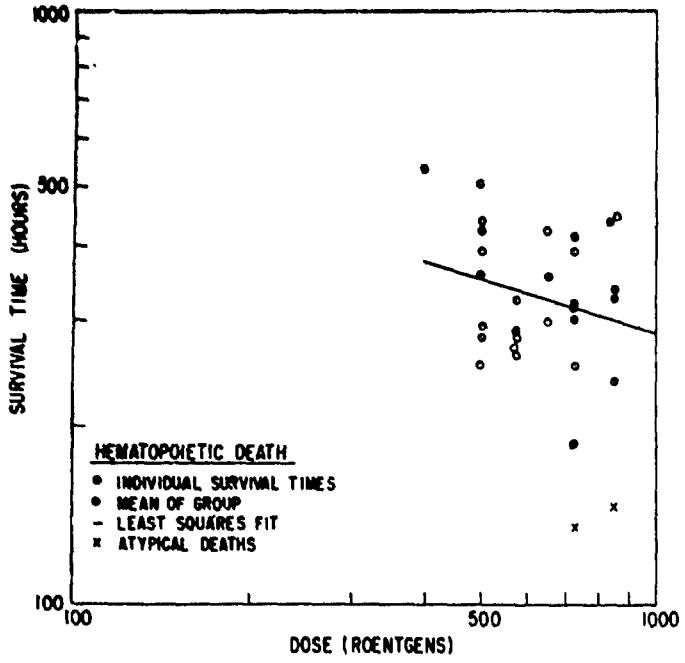

FIGURE 7

Survival time in low-dose range.

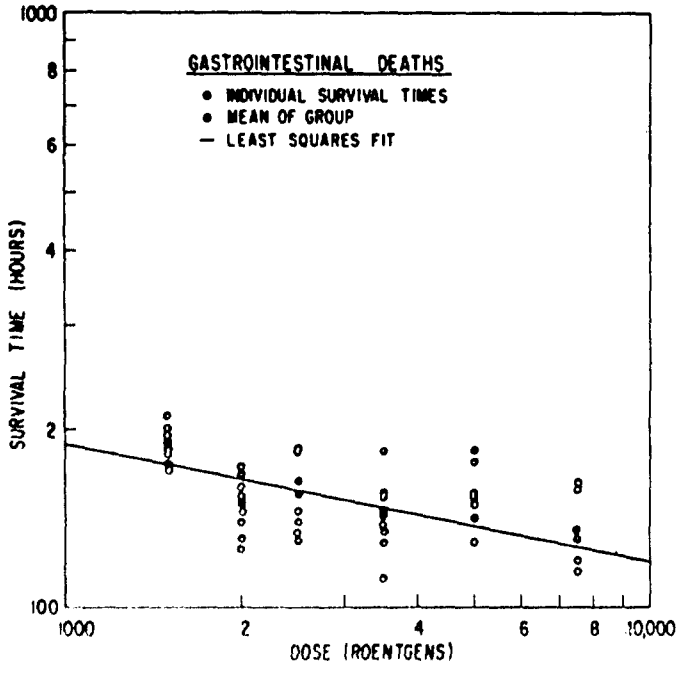

FIGURE 8

Survival time in mid-dose range.

also gave a satisfactory fit; that is, the deviations from the zero slope line were within sampling error. The sums of squares of deviation from the fitted line for the linear and exponential curves were very nearly the same—one curve fitted the data as well as the other. For this range of doses, not all animals died within 30 days.

An $LD_{50/30}$ value of 438 r was calculated, using the technic of probit analysis. The $LD_{50/30}$ value has an estimated standard deviation of approximately 89 r, giving an approximate 95 percent confidence region of 260 to 616 r. The reason for this very large imprecision in confidence limit estimation is the failure, in this dose range, of the percentage mortality to increase uniformly with increasing dose. This observed reversal in percentage mortality is probably attributable to sampling fluctuation owing to the small sample sizes used in estimating the percentage mortality.

The middle portion of figure 10 is designated as the region of gastrointestinal death. The linear equation

$$\log ST = 2.868 - .1999 \log D$$

was fitted to these data. The curve was an adequate fit and was significantly better than the mean line ($P < .01$). An exponential curve was not fitted in this region.

The right hand plot of figure 10 consists of data relating to central nervous system type of death. The linear curve

$$\log ST = 8.568 - 1.750 \log D$$

is shown as an unbroken line, and the exponential curve

$$\log ST = \exp (6.652 - 1.551 \log D)$$

as a dashed line. Both curves are adequate fits and were significantly better than the mean line ($P < .01$). The sum of squares of deviations from the exponential curve was only about one-half the sum of squares of deviations

from the linear curve. This suggests that the exponential curve is a better fit than the linear model for this sample. At present, however, there is no way of testing which curve is the better approximation to the true curve, since they both fit with sampling fluctuations.

CLINICAL OBSERVATIONS

The clinical picture exhibited by the exposed animals represented a panorama of symptomatology that can be observed only when a large population is irradiated by such a wide gamut of total body doses. The species of laboratory animal chosen for this experiment approaches closely the human being in physiology, anatomy, and pathologic response. Indeed, the clinical course followed closely that of human beings who have been exposed to ionizing irradiation accidentally, or in the employment of nuclear weapons in warfare.

During the course of these observations, it became apparent that certain groups of symptoms usually occurred together, and we have categorized them as follows: (1) central nervous system — meningismus, ataxia, nystagmus, tremors, convulsions, and hyperirritability in any combination; (2) gastrointestinal system — vomiting, anorexia, diarrhea; and (3) hematopoietic system — signs of increased bleeding tendency, septicemia, skin infections, and ulcerations. In addition, some 70 percent of all the animals exhibited a symptom complex we have called debility, consisting of an extreme loss of interest in all surroundings, disinclination or inability to move, and a tendency to sit, unmoving and huddled over with a bowed head and ruffled fur, in a remote corner of the cage.

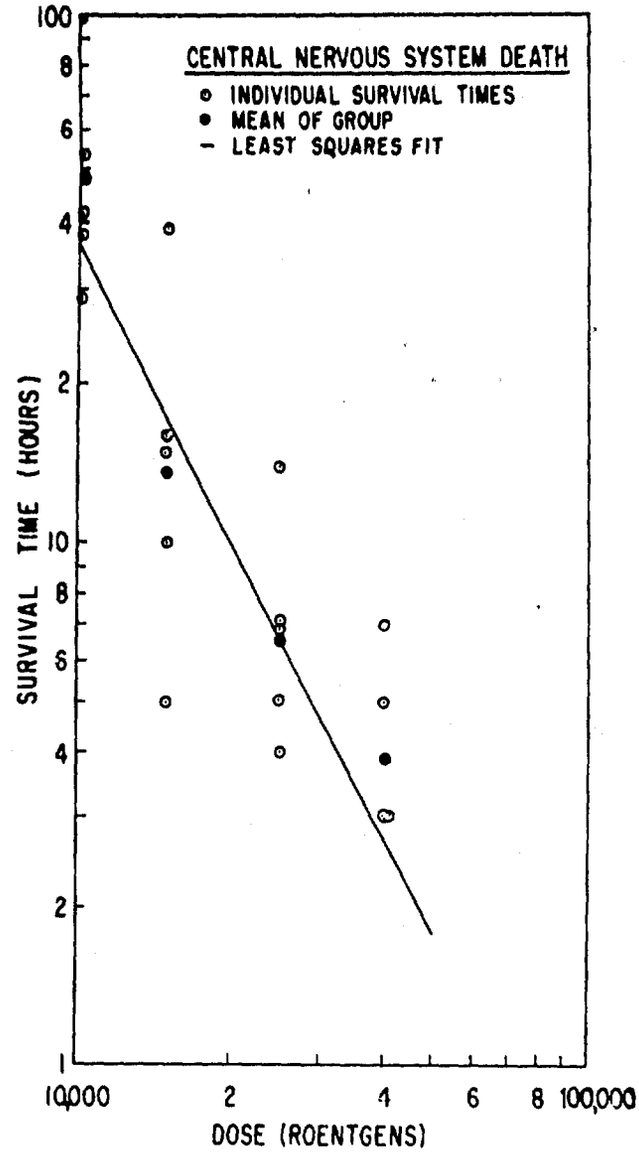

FIGURE 9

Survival time in high-dose range.

It would serve no purpose to present in a detailed fashion the clinical findings in each animal, or even each dose group of animals. However, one can distinguish in this experiment three patterns of clinical response to irradiation. It is remarkable that the animals within respective dose groups exhibited such uniform symptomatology. There was some individual variation in these manifestations, particularly in the lower dose ranges, and a very few animals succumbed without any significant symptoms. If one compares the patterns of clinical behavior with day of death rather than with dose groups, the resulting grouping shows no greater homogeneity.

Central nervous system pattern

During irradiation, a few of these animals exhibited hyperirritability and scratching movements at about 3 to 4 minutes (3,000 r). This rapidly subsided, and then all animals entered a period of progressively severe debilitation at

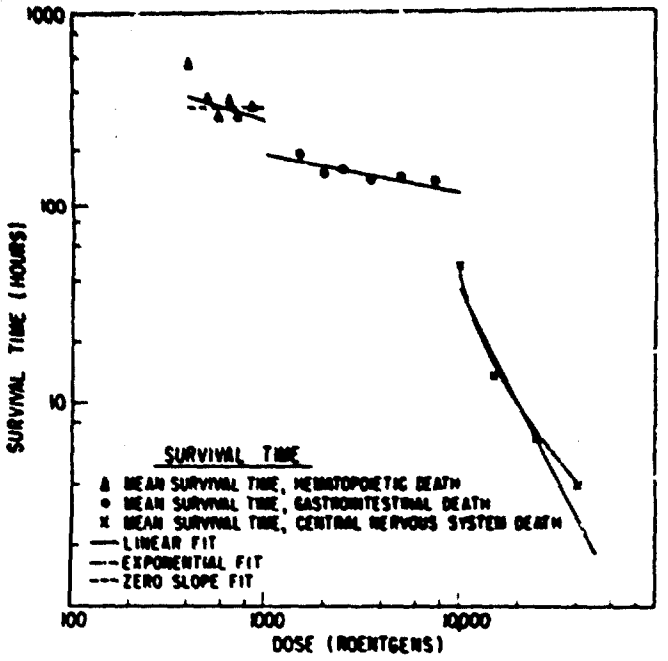

FIGURE 10

Survival time of entire range of doses.

about 8 to 10 minutes (7,000 r). A few approched a semicomatose condition without any evidence of ability to support themselves within the cage.

Approximately one-third of the animals had salivation, mouthing, or vomiting during irradiation, and a few had watery diarrhea. The incidence of vomiting is related to preirradiation feeding, and since animals were purposely fasted 8 hours prior to irradiation to facilitate their handling, the low incidence of this phenomenon could be expected. Some of the animals demonstrated nystagmus during irradiation, and suffered from ataxic movements and convulsive seizures. Following irradiation, these animals had progressive symptomatology as described above, with the addition of meningismus as shown by a markedly opisthotonic posture, and tremors which were difficult to distinguish from convulsions and ataxia. The animals developed the symptoms to a degree and with a rapidity which was dependent on the total dose received. For hours before the heart ceased to beat in these animals, complete loss of consciousness, opisthotonus, spasmodic gasping respiration, persistent tremors, convulsive movements, and often diarrhea, were the common clinical observations. The time of death was computed for statistical purposes as the cessation of heart beat. This pattern was found in an advanced stage almost universally and solely in the 9,000 to 40,000 r range. Death occurred in this group within the first 3 days.

Gastrointestinal pattern

None of these animals exhibited hyperirritability during or after irradiation. In certain dose groups where the time of exposure was appropriate for unimpeded observation, a characteristic biphasic debilitation occurred. In this situation, the animals were completely prostrated about 10 minutes after irradiation, and then would recover to apparent complete normality in about 30 to 60 minutes. In the succeeding hours of observation, moderate degrees of debilitation recurred. In a few animals this persisted until death, while in others it disappeared after 10 to 12 hours and reappeared terminally. This early debilitation occurred in nearly all of these animals, although only the second phase was frequently observed because of obscuration by the exposure cage in the longer exposures, and because the initial phase did not appear below 2,500 r. All animals demonstrated prostration terminally.

All animals in the higher dose range of this group exhibited central nervous system symptoms, but they were milder and were transient. At the lower doses debility seemed to be the predominant early symptom, although a few animals exhibited even milder tremors and ataxia.

The incidence of early diarrhea and vomiting was not significantly different in these animals as compared to the high dose group. Following the initial clinical findings described above, all of the animals returned to normal.

Partial or complete anorexia and diarrhea began on the 5th postexposure day. In this group of animals, diarrhea was seen nearly universally, and anorexia was prominent in over three-fourths of the subjects. When weighed at 5 days, an associated body weight loss of about 15 percent was present and this increased to 20-25 percent at 10 days.

Thus, we can summarize this group which was found in the 1,000 to 7,500 r dose range and died on the 5th day through the 9th day after irradiation: Early debility and central nervous system symptoms were transient, with recovery. Following this period of transient debility, anorexia and diarrhea became the prominent clinical findings. These two findings were evident on the 5th postirradiation day and persisted until the time of death. At the time of death the animal was listless and wasted.

Hematologic pattern

Again, an appreciable incidence of mild central nervous system symptoms and debility was observed in the immediate postirradiation period, with subsidence and disappearance of these symptoms in less than 24 hours.

The occurrence of vomiting and early diarrhea, however, was markedly reduced as compared to the previous two groups. Again, beginning on the 5th postirradiation day, anorexia and diarrhea in a less severe form was noticed in some of these animals.

The anorexia and diarrhea disappeared in most of the animals who subsequently died with other symptoms. In others, anorexia and diarrhea abated temporarily about the 8th to 10th day and then recurred, and in some animals persisted until death. Occasionally in these animals, no other symptoms appeared before death on the 10th to 22d day. These animals were classified clinically as gastrointestinal deaths.

Beginning on the 11th postirradiation day, evidence of decompensation of the bone marrow began to appear in the group. This consisted of hematoma formation, purpura, petechiae, hemarthroses, epistaxis, gastrointestinal bleeding, and ulcerations, particularly about the face.

Additional prominent clinical findings in this group were local skin changes. To a lesser extent, the same findings were observed in the previous group of animals but at a time later in their postirradiation course. Erythema especially about the face began on day 5, occasionally progressing to edematous swellings with ulcerations. Epilation began on day 12 and persisted for the rest of the experiment. All of the late findings of bone marrow depression and skin changes occurred in 25 to 50 percent of the low dose group, depending on the parameter measured. Because of the small size of the experimental groups, the actual numbers involved are of questionable significance.

This hematologic pattern was found in the animals comprising the dose range of 400 to 1,000 r and death occurred on the 10th to 23d day. No deaths occurred after the 23d day in the 6-month period following irradiation.

Clinical findings in the eleven 30-day survivors

These animals revealed a lower incidence of the central nervous or gastrointestinal symptoms, and a higher incidence of hematologic and skin abnormalities, as compared to the entire exposed group. This might be expected, because the survivors were part of the 400 to 600 r dose range. Close study of the clinical records reveals that the signs were mild and transient as compared to those observed in members of their respective groups. Maximum weight loss of 20 percent initial body weight occurred in these animals on the 17th day, with a subsequent return toward normal.

Figure 11 graphically represents the incidence of the symptoms observed in the various dose ranges. In figure 12, the occurrence of clinical symptoms is depicted with reference to their temporal appearance in the surviving subjects.

EVALUATION OF GROSS AND MICROSCOPIC PATHOLOGY

During the analysis of the gross and microscopic autopsy material from these animals, it became apparent that three basic pathologic patterns existed. Whether these patterns are classified as *early*, *intermediate*, or *late* with

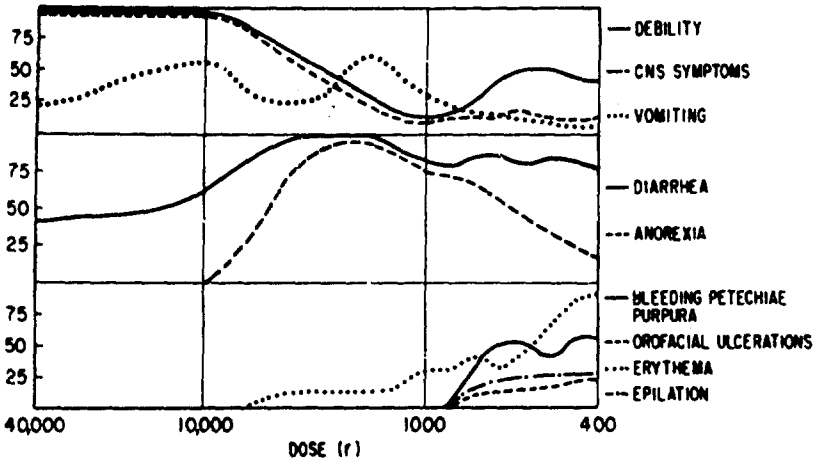

FIGURE 11

Prominent clinical symptomatology in various dose ranges.

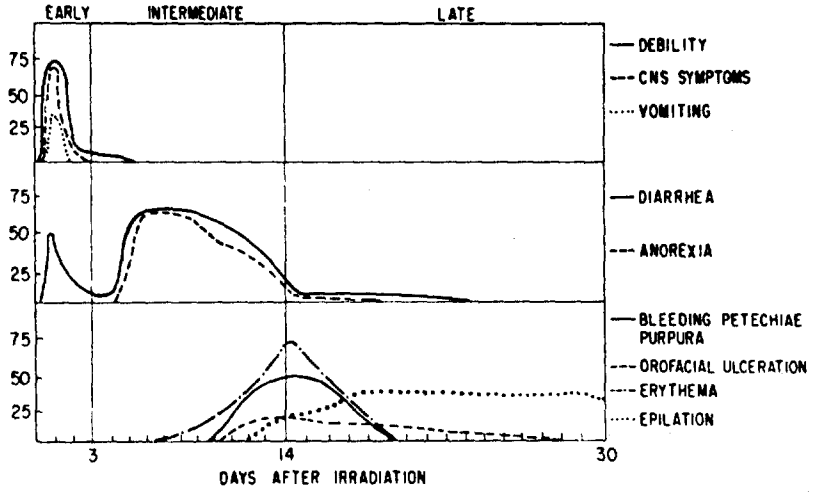

FIGURE 12

Occurrence of clinical symptoms with respect to time after irradiation.

regard to time, or as *central nervous system, gastrointestinal,* and *hematopoietic* with respect to clinical symptomatology makes little practical difference. Since the mechanism of acute radiation death is so complex, it would be imprudent to assign a single, specific cause of death in many instances. The hematopoietic deaths that occur at relatively low doses late in the course of acute radiation disease are a possible exception to this statement.

It is desirable to maintain brevity in using the anatomic material in this text. Since large numbers of animals are involved, only major pathologic findings will be discussed. Intricate microscopic descriptions will be deleted where suitable descriptions of similar lesions preexist in the literature. Attention will be focused on the central nervous system, the gastrointestinal tract, the bone marrow, and the lymphoid tissue. Reference will be made to other organs and systems only when it is deemed absolutely essential for completeness of the pathologic description.

The autopsy technic was designed so that a single case could be completed within 20 to 25 minutes. In the high-dose groups, the brain was removed first, because the prime objective was to place the tissue into the fixative solution as soon as possible. Small wedges from each organ were placed in Bouin's solution. Multiple slices were made through the remaining portions of the viscera and all of these were preserved in neutral 10 percent phosphate buffered formalin. Sections were cut at 4 microns in 62° paraffin and stained with hematoxylin and eosin.

After the histologic material was prepared, each case was interpreted without full knowledge of the clinical course and total dose of radiation received. After these impressions were recorded, the slides were rearranged so that the animals could be compared by systems and organs; that is, each slide was graded according to degree of damage with respect to the appearance of similar slides from all of the other animals.

Early Deaths

Gross findings

As might be expected following such rapid death, there were relatively few gross abnormalities found at autopsy. The most consistent feature was meningeal congestion, and this varied markedly in its severity without regard to time of death or total dose received. Seven of the 20 animals had noteworthy pleural effusions and pulmonary edema. In 3 of the animals that died near the end of the 1st day and on the 2d and 3d day there were petechiae in the colonic mucosa.

Microscopic findings

Central nervous system. Each of the animals had some degree of subarachnoid perivascular hemorrhage. This finding has been observed in nonirradiated animals and its significance is not known. In some cases there were only a few erythrocytes surrounding the vessels. In 13 animals there were foci of cerebral vasculitis and focal, acute meningitis (figs. 13 and 14). There was extensive pyknosis of the granular cell layer of the cerebellum and the pituitary basophils as described by Haymaker et al. (8) (figs. 15 and 16). The 4 animals receiving 10,000 r and the 1 animal receiving 5,000 r were less severely affected than the remainder of the animals.

Lymphoid tissue and bone marrow. In the last 5 animals to die, the spleen, lymph nodes, and bone marrow were practically devoid of their characteristic cells.

Gastrointestinal tract. Two interesting features which will be mentioned only in passing are degeneration of parietal cells in the gastric mucosa, and degeneration of the islet cells of Langerhans.

The acute degeneration of parietal cells was extremely marked in several of the high-dosage animals. It was characterized by marked

FIGURE 13

Veins in leptomeninges of animal dying 9.8 hours after receiving 15,000 r. Neutrophils infiltrate all portions of the vessel walls. Hematoxylin and eosin stain. (245×)

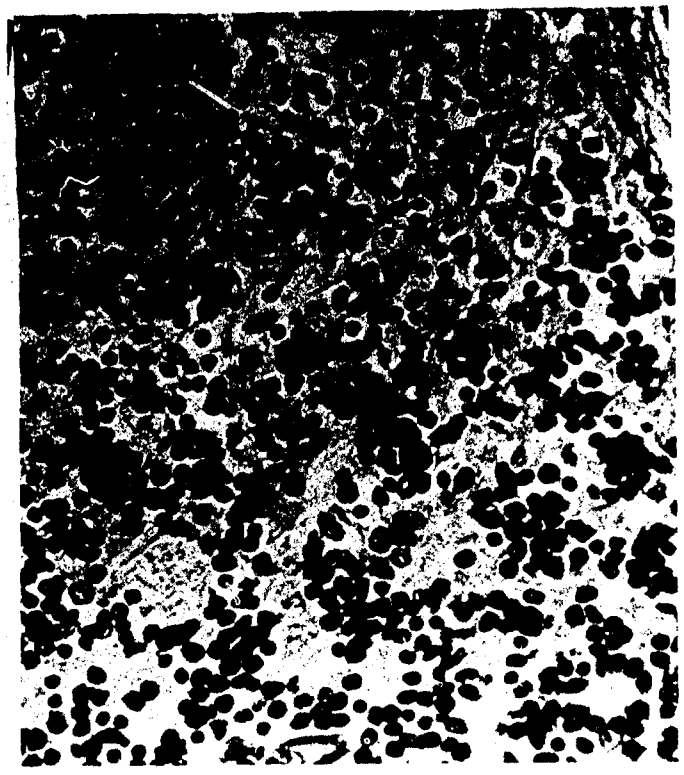

FIGURE 15

Pyknosis of granule cells in cerebellum of animal dying 5.5 hours after receiving 40,000 r. Nuclei are markedly decreased in size and extremely dense. (400×)

FIGURE 14

Generalized neutrophilic infiltration of leptomeninges with extension into Virchow-Robbins space. From animal dying 6.6 hours after receiving 5,000 r. (245×)

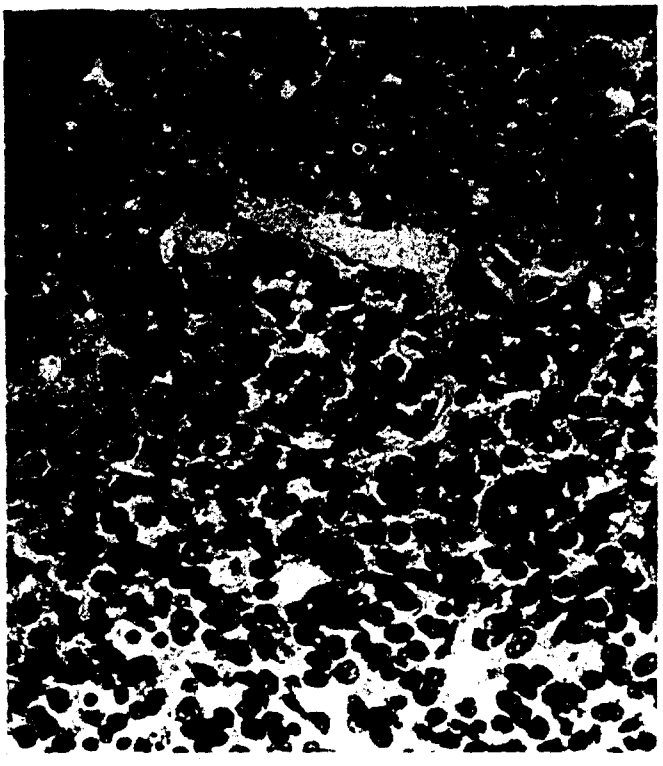

FIGURE 16

Typical case of pyknosis of basophils in pituitary of animal dying 6.5 hours after receiving 25,000 r. (250×)

nuclear pyknosis and extreme eosinophilia of the cytoplasm. Clusters of degenerating cells could be found floating free in the lumina of the gastric glands. At times the changes involved practically every parietal cell while on other occasions only scattered cells or groups of cells were involved. In almost every instance, the superficial mucosa epithelium was histologically normal (fig. 17). Necrobiosis of the chief cells was observed in only 2 animals.

The degeneration of the islets of Langerhans was also quite pronounced. At times, entire islets were necrotic; however, scattered throughout the same pancreas would be intact islets. On occasion only scattered cells in the islet would be undergoing degeneration (fig. 18).

Other changes in the gastrointestinal tract were limited to scattered nuclear atypism and increased nuclear dust in the basilar portions of the small intestinal glands, the colonic glands, and the squamous epithelium of the tongue and esophagus.

Intermediate Deaths

The earliest death in this group was on the 4th day. The latest death was on the 9th day. In all, there were 44 animals in this group and they received between 1,500 r and 7,500 r. One animal received 10,000 r. There were 3 animals that received less than 1,500 r, but these will be treated separately.

FIGURE 17

Gastric mucosa of animal dying 2.8 hours after being irradiated with 40,000 r. Note that mucous cells of superficial mucosa are intact. Parietal cells are degenerating. Note pyknotic nuclei. Cytoplasm was markedly eosinophilic. (250×)

FIGURE 18

Extremely severe nuclear pyknosis of all the secretory cells in an islet of Langerhans. Monkey expired 6.8 hours after receiving 40,000 r of whole-body gamma radiation. (370×)

These deaths were initially separated from the early deaths on the basis of the microscopic material alone. However, the conclusions were almost exactly the same as those reached by subsequent evaluation of the gross autopsy material.

The initial dividing line chosen was the development of cellular atypism in the small intestine and colon (fig. 19). Every intermediate death animal exhibited this change while animals in the early and late deaths did not. There was a certain degree of nuclear atypism in the intestinal tracts of the first 20 animals to die, but there was a marked difference between the somewhat mild nuclear atypism seen in the early deaths and the prominent cellular atypism seen in the intermediate deaths.

FIGURE 19

Typical cytologic atypism of epithelial cells seen in small bowel and colon in the intermediate death animals. The nuclei are bizarre and there are occasional multiple nucleoli. There is little mucous production and in most instances the cytoplasm has lost its cuticular border. From animal which died 6 days postirradiation. Total-body irradiation with 2,000 r. (430×)

Gross findings

Congestion of the superficial meningeal vessels was also prominent in this group of animals. However, pleural effusions and pulmonary edema was virtually absent. The most characteristic finding was moderate to severe ulceration of the mucosa of the cecum and ascending colon (fig. 20). Severe gastric ulceration was prominent in those animals dying on the 4th through the 7th day; thereafter it disappeared. In almost every case it involved only the body of the stomach (fig. 21). Laryngeal obstruction secondary to hemorrhagic ulcerations in the larynx was observed occasionally. It is well to note at this time that the laryngeal ulceration in these animals appeared to have a different pathogenesis from that observed in the animals in the 400 to 850 r dose range. The former evidently resulted from the abnormal growth of the epithelial cells, and hemorrhage resulted from the ulcerations. Conversely in the latter it appeared that localized hemorrhage probably resulted in mucosal ulceration.

Microscopic findings

Gastrointestinal tract. The areas of ulceration in the colon and stomach were generally superficial. In most cases there was simple hyalin necrosis of the mucosal elements with shadowy outlines of glands and lamina propria remaining. Covering the ulcerated areas was a firbinous membrane generally containing large numbers of bacteria. It is interesting that even though the small intestine exhibited the same degree of cytologic atypism as the colon, there were no ulcers in this portion of the gastrointestinal tract. Indeed, there was only a rare focus of hemorrhage. There was no islet cell degeneration.

Bone marrow. In every case there was severe atrophy of the bone marrow. The predominant cells remaining were plasma cells and reticulum cells.

FIGURE 20

Cecum of animal dying on 5th day postirradiation. Mucosa superficially ulcerated and covered by fibrinous membrane containing colonies of bacteria. Small intestinal epithelium and the intact portions of the colonic mucosa exhibited cytologic atypism similar to that in figure 19. Total dose, 7,500 r.

Lymphoid tissue. Severe atrophy of all lymphoid tissue was a constant finding.

The central nervous system was devoid of any changes except for the aforementioned subarachnoid hemorrhage of questionable significance.

Late Deaths

By far the greatest array of abnormal anatomic findings was in the animal receiving 850 r and below. However, the bulk of the findings were attributable to the effects of local bacterial infiltration and/or hemorrhagic phenomena. This group of 24 animals received between 400 r and 850 r. All of the deaths occurred between the 9th and 23d day.

Gross findings

On the 9th through the 11th day, findings at necropsy were limited for the most part to scattered gastric and skin petechiae. During the 12th to the 18th day, however, the findings became much more severe and widespread. They included serosal surface hemorrhages, edema of the face, lips, soft tissue of the neck and lungs, skin petechiae, and hemorrhages into the pleural cavities (figs. 22, 23, and 24).

Microscopic findings

Heart. In 3 animals, the myocardium contained scattered foci of bacteria. As a general rule there was necrosis of the myocardial fibers immediately surrounding the bacterial colonies.

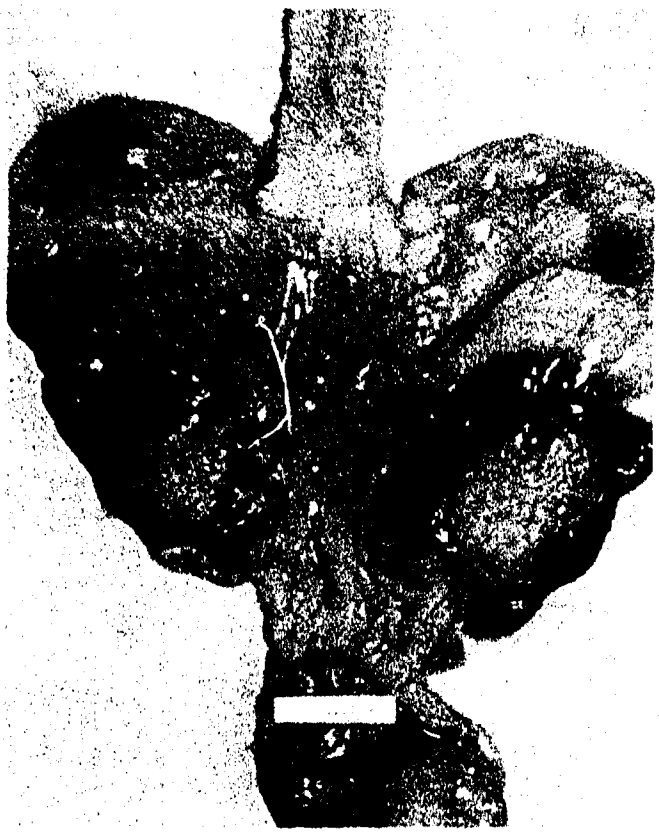

FIGURE 21

Typical gastric ulceration occurring in the intermediate deaths. In practically every case only the body of the stomach was involved. Occasionally ulceration involved the entire circumference of the stomach. From animal dying on 5th day after being irradiated with 7,500 r.

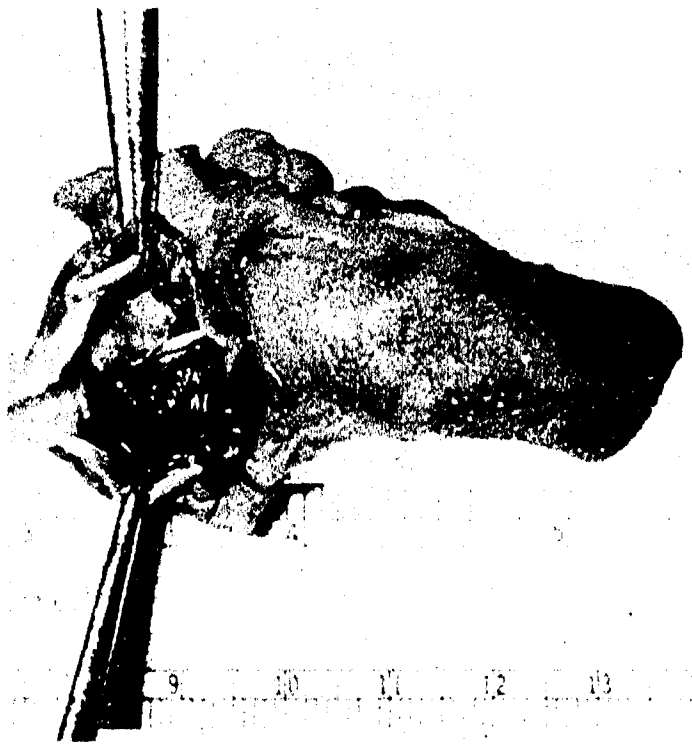

FIGURE 22

Hemorrhage completely obstructing larynx of animal expiring 13 days after being irradiated with 725 r.

Spleen. In every case the spleen was severely atrophic, and one contained massive colonies of bacteria.

Lungs. Nine of the animals exhibited extensive bacterial growth throughout all portions of the lungs. Often the bacterial growths were associated with localized hemorrhages. In three cases there was moderate pulmonary edema.

Liver. When there was other evidence of generalized bacteremia, there was also bacterial infiltration of the liver sinusoids.

Gastrointestinal tract. Even though colonic ulceration was rather frequent, and at times severe, the microscopic and gross appearance of these lesions was entirely different from that observed in the intermediate deaths. In contrast the ulcers were often deeply penetrating. The muscular layers were often involved. In several of the animals there was perforation of the gut wall with resultant peritonitis. In addition, the ulcers were generally discrete. The predilection for the cecum was not as pronounced as in the intermediate deaths. There was no cytologic atypism of the epithelium of the colon or small intestine.

Several animals in this dose range that were sacrificed at 68 hours, 6 days, and 9 days showed no epithelial atypism in the gastrointestinal tract.

Lymph nodes. There was severe atrophy of the lymph nodes. Where the nodes were draining an area of bacterial infiltration, they were often markedly congested. Those draining the colon and the oral cavity were frequently infiltrated by bacteria.

FIGURE 23

Massive intracavitary hemorrhage in 650 r animal dying on 17th postirradiation day.

FIGURE 24

Typical gingival ulceration frequently found in all of the animals receiving 850 r and below. Note small, superficial ulceration on chin. From animal dying on 14th day after receiving 575 r.

Bone marrow. All bone marrow was practically acellular. There were occasional plasma cells and reticulum cells, and in the animals dying after the 16th day there were rare foci of early regeneration.

Atypical Deaths

There were 3 animals that expired during the intermediate death period that received less than 1,500 r. Two were in the 725 r group and 1 was in the 850 r group.

Two of the animals died identical deaths. Each had severe hemorrhagic cystitis with squamous metaplasia of the bladder mucosa. There was severe hydroureter and hydronephrosis with severe renal cloudy swelling. The third animal received 725 r and died on the 8th day. The autopsy findings were similar to those formed in the animals dying on the 9th and 10th days. Anemia and congestive failure probably played major roles in the demise of the animal.

Summary of Pathologic Observations

Widespread necrosis of individual cells without breakdown of basic tissue structure was one of the most dramatic features of the first 20 animals to die. Equally prominent was the severe damage to the cerebral vascular system and the pulmonary vessels. These features, together with the absence of cytologic atypism in the gut epithelium, set this group of animals apart from all of the remaining animals of this

series (see figure 25). All of these animals died during the first 3 days.

The intermediate death animals could be sharply divided from the remaining two types of deaths observed in this series. The marked cellular atypism observed in the small intestine and colon undoubtedly severely affected the physiology of the animal. This change had a sudden onset beginning on the 4th day and was not found in any animal receiving less than 1,500 r (fig. 25). There were moderate differences in the morphology of the lesions in the 1,500 r animals as compared to the 2,000 r animals. No significant differences were noted in any of the higher-dose animals of this intermediate group.

Gastrointestinal tract changes were not limited to microscopic findings. The incidence of gastric ulcerations and colonic ulcerations was extremely high. One of the most interesting phenomena observed was the absence of ulcerations in the small intestine even though the cytologic changes in the small intestinal epithelium were identical to those observed in the colonic mucosa.

The limitation of gastric ulceration to the body of the stomach and the marked predilection of the cecum and transverse colon for ulceration are probably closely associated with cell function. These two findings are the subject of current investigation in this laboratory.

In the first few animals dying in the late death group, there was a dearth of anatomic findings. It appears probable that these animals died of severe anemia, resultant congestive failure, and cerebral anoxia. As the deaths progressed into more severe stages of bone marrow failure, however, overwhelming infection became obvious. Seldom was there a case without some degree of bacteremia. In most instances several organs were infiltrated by bacteria; other frequent findings were pulmonary hemorrhages and occasional small hemorrhages in critical structures such as the larynx. The vast majority of the animals died of bone marrow failure as manifested by bacteremia and hemorrhagic phenomena.

Colonic ulceration was frequent in the late deaths, and it often mimicked the lesions observed in the intermediate deaths. It should be emphasized, however, that the two lesions are entirely different when closely examined. The ulcers in the late deaths were primarily due to bacterial infiltration. There was no epithelial atypism. Also the lesions were often deeply penetrating. In contrast, the ulcerative lesions in the intermediate deaths were generally superficial and apparently due to primary cell death as a result of irradiation.

DISCUSSION

The three approaches to this experiment — namely, statistical evaluation of survival time, clinical observations, and pathologic evaluation –

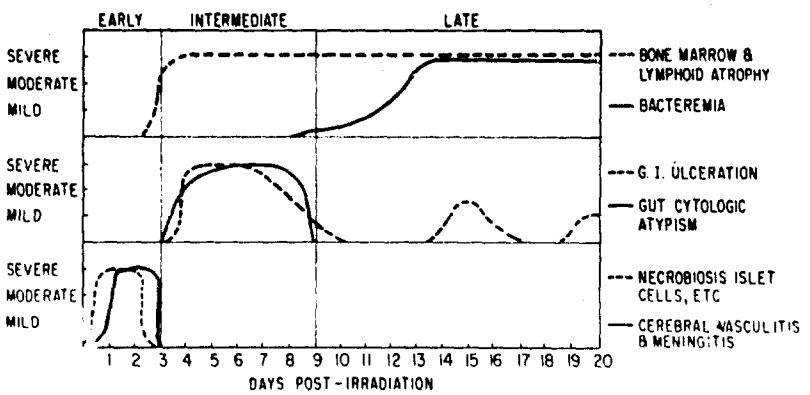

FIGURE 25

Severity of pathologic findings according to time of death.

have individually led to similar conclusions with regard to mechanisms of radiation death associated with three dose ranges.

In the high-dose groups (9,000 to 40,000 r). the predominant clinical pattern was one of severe and progressive dysfunction of the central nervous system. It is our conviction that injury to all other tissues was severe but clinically less impressive. The original classification of this mechanism of death has been described accordingly as a central nervous system death. The pathologic observation of the somewhat subtle findings in nervous tissue is probably related to inadequate time and ability for tissue response to injury, and does not reflect the severe functional derangement. The equally visible changes in the gastrointestinal, respiratory, and hematopoietic organs were evidence of widespread injury which undoubtedly was the actual cause of death or at least markedly contributed to the demise of the animals. Since massive radiation produces generalized "biochemical and metabolic paralysis" or death of numerous cells, it would be reasonable to expect a relationship between dose and survival time. The statistical evidence obtained in this study confirms this concept, with an exponential relationship between dose and survival time. The characteristic clinical and pathologic pattern in these animals correlates with this region of the discontinuous survival curve.

In contrast, those animals comprising the intermediate dose groups (1,000 to 10,000 r) are distinguished by a linear log survival – log dose relationship, which is less dose dependent. This, too, was associated with characteristic clinical signs and pathologic changes. While anorexia and diarrhea are not necessarily specific manifestations of radiation injury, the pathologic changes observed can be considered as highly specific for radiation induced pathology. The cellular atypism, and type and distribution of gastrointestinal ulcerations have not been observed from any other etiologic agent.

The absence of hemorrhage and sepsis in this group in the face of bone marrow atrophy was presumably due to the fact that residual circulating bone marrow elements protected the animals from such events until the 3d week postirradiation.

The threshold for the specific mucosal damage as manifested by cellular atypism appears to lie between 1,000 and 1,500 r. This pattern of response appears to be nearly dose-independent, until sufficiently high doses are reached at which significant abnormalities in other organ functions are produced. Previous communications have emphasized the importance of denudation of the gastrointestinal mucosa in this mechanism of death. We feel, however, that the more basic change is represented in the appearance of atypical, abnormal appearing cells which have lost their normal functional capacity. These represent the locus of subsequent ulceration and denudation only when combined with bacterial activity. This probably accounts for the conspicuous absence of ulcerations of the small intestine in these monkeys. However, deficiencies in absorption of nutrients from the lumen, loss of the protective barrier against bacterial invasion, and electrolyte imbalance may explain the mechanism of death before ulceration occurs, purely on the basis of these atypical cells.

In the lower dose groups of 400 to 1,000 r, marked variations in the clinical syndrome and pathologic findings were observed, but the common denominator was universal failure of the bone marrow. By far, the greatest number of deaths could be attributed to bacteremia and localized destruction of tissue by bacteria proliferating in the absence of host resistance. Occasionally, localized hemorrhages compromised vital functions, and led to rapid demise.

The group of animals, as a whole, presented difficulty in prognostication. A minor insult is sufficient to produce extensive injury and death because of decreased resistance to infection. The scatter of points representing survival time versus dose may be explained at least partly on this basis.

In some of the animals in this dose range, differences in clinical and pathologic diagnoses appeared. These animals exhibited continuous diarrhea without bleeding or infection until the time of death. Pathologically, however, the gastrointestinal changes represented secondary bacterial ulcerations rather than the primary type of gastrointestinal injury characterized by atypism of mucosal cells and shallow ulcerations.

The spacing of the experimental groups as utilized in this experiment precluded the occurrence of significantly overlapping, clinicopathologic syndromes. It is to be expected, however, that transitional zones would occur with appropriate grouping. Three cases which demonstrated atypical deaths with respect to time, clinical signs, and pathologic findings had pre-existing disease. One 5,000 r animal died at 6 hours with classical central nervous

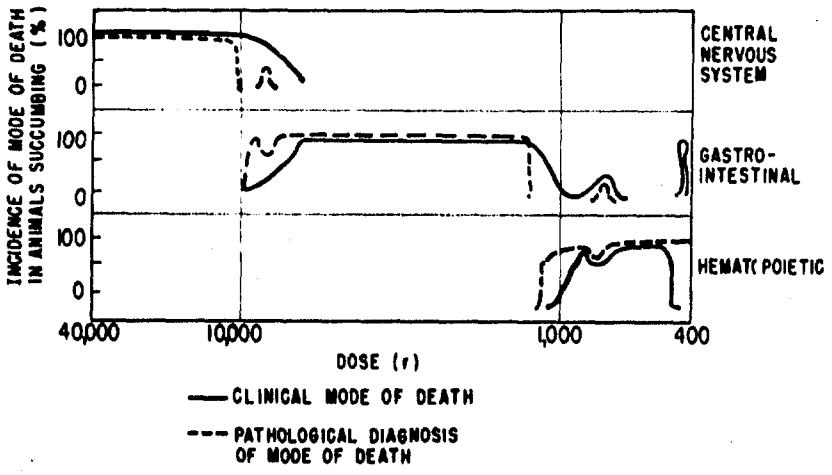

FIGURE 26

Clinical and pathologic mode of death by dose.

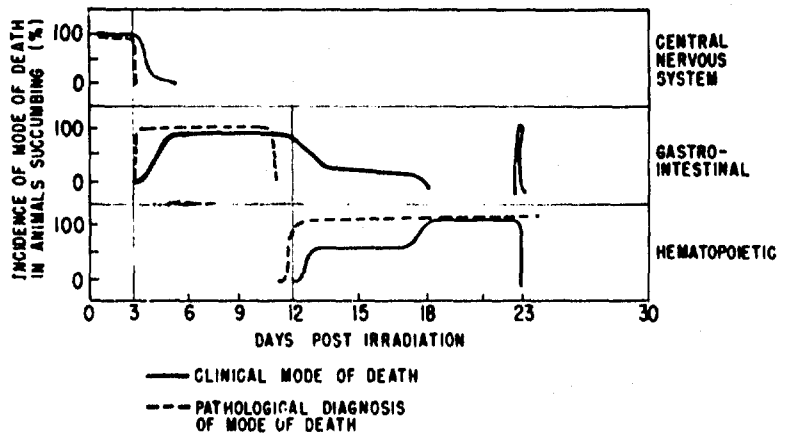

FIGURE 27

Clinical and pathologic mode of death by time of death.

system symptomatology, but it has been observed that doses of radiation much smaller than 5,000 r have produced severe changes in the nervous tissue (8). Figures 26 and 27 demonstrate the close correlation of clinical and pathologic classifications of modes of death when plotted against dose ranges and time of death.

In evaluating the injury pattern and mechanism of death following radiation, one must consider the influence of degree of injury, and rate and degree of repair, as well as individual specific tissue radiosensitivity and latent periods for manifestations of injury or response. Predisposing lesions and other extraneous factors can strongly influence these relationships.

SUMMARY

One hundred seven *Macaca mulatta* monkeys were exposed in 16 groups to rapid delivery of Co^{60} gamma rays at doses of 400 r to 40,000 r.

Statistical evaluation of the survival time determination of the $LD_{50/30}$ dose, clinical observations for a 30-day postirradiation period, and immediate postmortem examinations were performed.

These three approaches to the investigation of the mechanism of radiation injury and death in the monkey were evaluated. Each method separated the groups of animals into three distinct and corresponding categories.

The clinical and pathologic studies undertaken in this experiment provide further elucidation of the central nervous system, gastrointestinal, and hematopoietic types of radiation death.

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